The Role of Repair in Environmental Mutagenesis

by William Gary Flamm*

Introduction

Gene repair is a unique phenomenon, or at least appears to be, since it connotes the "intelligent" repair of a biomacromolecule. The molecule in question is, of course, DNA and there are compelling reasons to believe that it is sound policy for a cell to provide a mechanism for the repair of damaged DNA. Indeed, the only alternative is to degrade and destroy the damaged molecule and attempt to replace it by *de novo* synthesis. But how, and what would serve as the blueprint; the template? Thus, for the problem and its solution, to maintain the blueprint which will inevitably be damaged in the course of time, repair for the DNA molecule must be available.

The questions which arise are: (1) how is gene repair carried out? (2) by what mechanism is repair mediated? (3) under what conditions is DNA repaired? (4) what kinds of damage are repaired? (5) does repair prevent cell death or cell mutation? (6) is repair always adequate or does it contribute to genetic alteration (mutation)? The following discussion attempts to deal with these questions to the extent possible, but the reader is reminded that the name given to gene repair was assigned long before much was known of the actual processes or their biological significance. When information was barely fragmentary, the meaning and significance of repair might have seemed more obvious than in light of additional facts and observations. Nevertheless, real progress is being made towards a fuller appreciation of the relationship between repair and the mutation process, and repair can be applied to a variety of important toxicologic problems of public health concern.

*Genetic Toxicology Branch, Division of Toxicology, Bureau of Foods, Food and Drug Administration, Washington, D.C., 20204.

Repair Synthesis in Bacteria

For many years it was understood that certain organisms recover either partially or completely from the effects of genetic damage. However, it was not recognized until recently that recovery, at least in some instances, is predicated on enzymically mediated repair of genetic damage. Of critical importance to this understanding was the fortuitous isolation of ultraviolet-sensitive mutants of *E. coli* B_{s-1} (*1*). Genetic analysis of these mutants proved that ultraviolet resistance is determined by three different genes mapping at widely separated loci within the *E. coli* chromosome (*2–4*). These sites have been designated as UVr, A, B, and C, and their mutants have been extensively employed to provide a biochemical understanding of repair processes.

In essence, these mutants served as controls in biochemical studies which showed that the excision of pyrimidine dimers (the lesions produced by irradiating DNA with ultraviolet light) from DNA could be correlated with the ability of cells to recover

from the effects of ultraviolet irradiation (*5*). Thus it became clear that UVr, A, B, and C genes were concerned with the excision of thymine–thymine and thymine–cytosine dimers, which is necessary to cell survival. In addition, it has been demonstrated that these repair-defective mutants are more sensitive to certain chemical agents than wild-type strains, particularly towards those agents capable of producing crosslinks in DNA (e.g., nitrogen mustard, mitomycin C, and nitrous acid) (*6–10*).

The first event in the repair of ultraviolet damage and presumably in the repair of chemically induced DNA crosslinks is excision of the offending lesion by an endonuclease (*11*). Excision is limited to one of the two strands of the DNA duplex so that lethal, double-stranded DNA breaks are avoided during the process of repair (*12*). Further degradation of the affected single strand proceeds following release of an oligonucleotide unit containing the original lesion (pyrimidine dimer or other crosslink). Approximately 30 nucleotides are removed per excised dimer. The resultant, single-stranded gap is repaired by replacing the original bases in correct order by Watson-Crick pairing with the bases on the opposite, intact strand. The final step in this process involves a ligase which catalyzes the rejoining of the single-stranded DNA break remaining after all the nucleotides have been replaced. This form of repair is now generally referred to as excision-repair or repair synthesis (*12*).

Other Types of Repair

Other types of genetic repair exist in *E. coli* as well as in many other organisms. Photoreactivation was among the earliest studied mechanism for the repair of ultraviolet damage to DNA. It occurs by direct enzymatic cleavage of pyrimidine dimer bonds upon exposure of cells to visible light (*13–15*). Information available at present indicates that this form of repair does not exist in mammalian cells. Recombinational repair is another form of repair which deals with ultraviolet-induced lesions in *E. coli*, though much less is known about its mechanism of action. Recombinational repair (*9, 16–19*) has been proposed to act by removing lesions which are too close to the replication fork to be dealt with by repair synthesis (excision repair) (*20*). Strand excision too near the replication fork might very probably lead to lethal damage equivalent to a double-stranded break in DNA. Hence, a mechanism is provided whereby lesions proximal to the replication fork are ignored by excision (repair synthesis) but are subsequently dealt with by recombination repair (following completion of DNA synthesis). This form of repair has also been termned post-replication repair.

It has not been definitively demonstrated whether mammalian cells engage in recombinational repair, and in fact, the main evidence in *E. coli* stems from the observation that this form of repair is governed by the locus controlling recombination (*16,17*). In addition, there is biochemical evidence supporting (*18*) the view that recombinational events provide a means of dealing with genetic lesions but this evidence is less convincing than that available on repair-synthesis.

Effect of Repair on Mutation

The importance to mutagenesis of having different repair processes operating in *E. coli* and other organisms concern their impact on cell survival and the induction of mutation (*21,22*). Indeed, it has been suggested that without recombinational repair, ultraviolet irradiation fails to induce mutations in *E. coli* (*23*). Presumably, ultraviolet mutagenesis, at least insofar as *E. coli* is concerned, is attributable to misrepair. On the other hand, available evidence (*23*) indicates that repair synthesis has much greater fidelity than recombinational repair and seemingly does not contribute to the induction of mutants. Thus, as outlined above, one process (repair synthesis) is responsible for repair of lethal damage and does not lead to mutation while another back-up or complementary process (repair-recombination) facilitates and mediates

mutagenesis though it also, under appropriate conditions, provides for increased cell survival (*19*). Whether misrepair contributes significantly to chemical mutagenesis is not known, but it is likely to be of great importance with respect to certain classes of chemical mutagens (e.g., bifunctional alkylating agents, compounds intercalated by DNA).

The consequences of repair or the lack of repair in terms of cell survival and mutagenesis are diverse and complex and are dependent upon the number and quality of genetic lesions and upon the relative activity of one form of repair as against the other. Thus, specific inhibition or interference with repair processes might lead to: (1) reduced cell survival with fewer mutations; (2) reduced cell survival with more mutations; (3) normal cell survival with fewer mutations. The first effect might be accounted for simply by nonselective inhibition of both repair synthesis and repair recombination. The second could be attributed to a selective inhibition of repair synthesis which could lead to greater recombinational repair of existent lesions and hence to the induction of more mutants. Specific inhibition of repair recombination under certain conditions (e.g., high ultraviolet levels and restricted DNA synthesis) might produce the third effect.

Clearly, an understanding of repair and how it interacts with and contributes to mutational processes has only begun. As alluded to above, most available information has been derived from repair-deficient mutants where "all or none" variations can be definitively examined. Such mutants provide a ready means of delineating the biological significance of a particular mode of repair with respect to specific conditions and genetic challenges. Repair-deficient mutants are not limited to *E. coli* or bacteria, but are known to occur in yeast, *Neurospora*, and even human individuals (*23*).

Repair in Mammalian Organisms

The hereditary disease in humans known as xeroderma pigmentosum is presently the only proven example of a repair-deficient mutant among mammalian organisms (*24–29*). It is an autosomal recessive disease characterized by the extreme sensitivity of skin to ultraviolet light. Skin fibroblasts from such patients generally exhibit reduced amounts of repair synthesis *in vitro*. As in the case of *E. coli* mutant, B_{s-1}, these mutant cells have afforded investigators the opportunity of determining some of the biological consequences of sub-normal repair synthesis both among cultured cells *in vitro* as well as in the individual. It has also been possible to determine the extent to which the mechanism responsible for the repair of ultraviolet lesions is involved in repairing chemical damage (*28*). For instance, it has been shown (*28,29*) that the carcinogen *N*-acetoxy-2-acetylaminofluorene produces lesions which are not repaired by xeroderma pigmentosum cells, while certain other chemicals and x-rays induce reparable damage. Perhaps of most importance, the mutant cells have provided greater confidence in the reliability of currently used analytical procedures for the detection and quantitative assessment of repair synthesis. In other words, trust in present methodology is predicated on an analogy with bacterial studies and upon experiments with cells derived from human mutants defective in repair synthesis (*24–26*). The present lack of mammalian mutants defective in recombinational repair continues to retard progress in this area, though there is evidence that two forms of DNA repair exist in mammalian cells following irradiation (*30*).

Methods of Assessing Repair in Mammals

Most recent efforts towards delineating practical and fundamental aspects of repair in mammalian systems have focused on repair synthesis because of the greater availability of procedures believed to be appropriate for the problem (*11,31–37*). There are in essence four different ways in which this form of repair can be assessed in mammalian systems and each procedure is related either methodologically or conceptually.

All the methods have in common the need to exclude normal semiconservative replication of DNA from consideration when assessing repair synthesis of DNA. Three of the methods do this by providing a means of resolving one activity from the other. The earliest procedure employed was termed unscheduled DNA synthesis and involves autoradiography of cytological preparations (*31–33*). Tissue culture cells are incubated for 1–2 hr in tritiated thymidine (^{3}H–TdR) prior to their exposure to the agent used for induction of repair synthesis. After this exposure, incubation in ^{3}H–TdR is continued for another 2 hr or more, and the cells are fixed and prepared for autoradiography. Heavily labeled cells represent those which were replicating DNA normally at the time of labeling and would have been labeled without exposure to the agent. The lightly labeled cells are in a different category; i.e., they are the cells which have been induced to perform repair synthesis by the inducing agent. Recent experiments have shown that this method can also be applied to the detection of repair in the germinal cells of rats treated with known mutagenic agents (*38*).

The most popular biochemical method for the assessment of repair synthesis of DNA utilizes bromodeoxyuridine (BUdR), which is an analog of thymidine and will replace it in DNA. This replacement produces, upon normal replication, DNA which exhibits an abnormally high buoyant density (*39*). Comparable buoyant density increases do not occur when BUdR is incorporated into DNA for the purpose of repair-synthesis (*40*). Thus, if radioactive BUdR is used or a combination of radioactive TdR and cold BUdR is employed, semiconservatively replicated DNA can be distinguished from DNA which has undergone repair synthesis on the basis of their differing buoyant densities. Resolution of such DNA's is readily obtained by centrifugation on CsCl density gradients (*39*).

The third and most recently developed method also incorporates BUdR, but in this case, semiconservative replication is distinguished from repair synthesis by an entirely different means (*29*). After BUdR is incorporated into DNA following the exposure of cells to an agent capable of producing reparable lesions, the DNA is subjected to irradiation with 313 nm light and placed in alkali. Breaks appear in the DNA at sites of incorporation of BUdR, causing the DNA to sediment more slowly in sucrose gradients. If DNA synthesis is due exclusively to normal semiconservative replication, a reduction in sedimentation rate is not observed.

All three of the above mentioned methods have relied heavily upon the use of repair-deficient, xeroderma pigmentosum cells as controls for the development of a suitable technique. More recently, attempts have been made to develop, refine, and apply a procedure wherein it is unnecessary to resolve the two types of DNA synthesis. This approach involves the total suppression of normal DNA replication, thereby providing assurance that incorporation of all precursors into DNA reflects repair synthesis only, uncomplicated by normal replication (*41–43*). Hydroxyurea has been used the most extensively for the purpose of inhibiting normal replication, though recently an artifact associated with its use in studying repair synthesis has been observed (*41*).

Certain *N*-hydroxy compounds which appeared to induce repair (i.e., hydroxylamine and *N*-hydroxycyclohexylamine) were found to interfere with the ability of hydroxyurea to suppress normal replication (*41*). Thus, the stimulation of DNA synthesis produced by exposure of these cells to *N*-hydroxy compounds was an apparent consequence of their interference with the inhibitory effect of hydroxyurea on semiconservative replication and not due to the ability of these *N*-hydroxy compounds to induce repair synthesis *per se*.

Application of Repair Studies

Despite the problems mentioned above, the hydroxyurea approach has been used successfully in a variety of repair studies

(*42–44*) and has the very great advantage of being highly sensitive, precise, and easy to apply. It has found current use as an indirect means of assessing genomal damage among human lymphocytes *in vitro*, particularly with chemical carcinogens and procarcinogens (*42,43*). As expected, the procarcinogens employed were inactive while carcinogens and mutagenic alkylating agents induced repair synthesis. Presumably, metabolic activation is required before procarcinogens are effective as inducers of repair. It would be of theoretical and practical interest to test the activity of procarcinogens *in vivo* by administering such compounds to an appropriate animal species and subsequently examining the corresponding lymphocytes for repair synthesis.

This approach need not be limited to procarcinogens either, for it could just as well be applied to promutagens; i.e., agents requiring metabolic activation for the expression of mutagenic activity. In one sense, this would be equivalent to a host-mediated assay and in fact could provide important complementary information as regards the mutagenic hazard of a particular substance —by indirectly assessing the extent of genomal damage produced.

One final point concerning repair synthesis should be mentioned and again it relates to what has been learned from studies with well defined mutants. Patients with xeroderma pigmentosum readily develop fatal skin cancers from exposure to sunlight and possibly to other agents which produce irreparable damage to DNA. Assuming, as seems reasonable based on genetic evidence, that repair synthesis in skin is related to carcinogenic events in that organ, this process would seem to be of critical importance, not only in controlling mutagenesis but also as a defense against the development of neoplastic disease. Thus, environmental agents to which man is commonly exposed would presumably have great potential for harm were they powerful inhibitors of repair synthesis. Accordingly, repair synthesis and environmental agents which might inhibit the process deserve further study from the point of view of public health concern.

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